

ANDREW GORBA:



EXHIBIT
1

Received well cord blood (w CPD)
PE monob 30%
T cells 70%

added 15 ml 1x PBS to bring to incubation volume of 45 ml.

Added 1.5 ml antibody (12.8)
incubated 25 ml.

Primed cell prep "ceprate".

spin cells. Resuspended in 1x PBS to a final volume of 300 cc in bag.

Ran through column.

Unadsorbed portion → spun down and consolidated in 1x PBS for incubation.

75 ml for incubation (added HSA)
1.5 ml antibody (12.8) 25 min. Spun down following incubation. Rsp'd to vol. of 300 cc in bag.
Ran through 2nd column.

stem cell portions from Runs 1 & 2 were combined (after counts done and samples removed for staining)

total cells 2.8×10^6 for transduction

BBmm +

1L3 SANDC

WEN

1L6 SANDC

WEN

SLF AUBIE

WEN

final concn

will be dil

cells are

concentrated

BBmm: FB

FB

2.8×10^6 cells

Put in

BBMM + 3161SCF (for 500ml of media)

123 SANDOZ * Y0230092 stock at 150ug/ml
want final: 20ug/ml x 2 ... 20ug

133ul add

126 SANDOZ * Y0450392 stock at 150ug/ml
want final: 50ug/ml x 2 ... 50ug

333ul add

SCF ANTIBEN * 1509F2 stock at 1.5mg/ml = 1500ug/ml
want final: 100ug/ml x 2 ... 100ug

167ul add

final concentrations are doubled since the media
will be diluted 1:2 w/ viral supernatant.
Cells are therefore incubated with the correct
concentrations.

BBMM: FBS Gemini lot# A76003H
BSA #115

2.8×10^6 cells want final: 5×10^4 cells.

Put in 2 T75 30ml each: 15ml B365 051193

15ml LPSU^{GT} lot# 53

+ protamine sulfate 240ul
of 1:20 diluted 50

in down
f 300cc in bag.

42
and

Cord Blood cells pre processing:

CPUs

SET 143

Start:

| | Plate # | Sample | # Cells | # ul/ml media |
|--------|---------|----------------|------------------------|---------------|
| - G418 | 1ab | Gdrea UCB ↓ | 5×10^4 ↓ | 50 |
| + G418 | 2ab | | | 50 |
| - G418 | 3ab | | 1.4×10^5 ↓ | 100 |
| + G418 | 4ab | | | 100 |

adsorb
fraction:adsorb
fraction

CPUs Post transduction: SET 144

| | plate # | # Cells | # ul |
|--------|---------|---------|------|
| - G418 | 1ab | 500 | 7 |
| | 2ab | 1000 | 14 |
| | 3ab | 2000 | 28 |
| + G418 | 4ab | 500 | 7 |
| | 5ab | 1000 | 14 |
| | 6ab | 2000 | 28 |

(yields)
adsorb

count:

$$\bar{x} = 34$$

$$1.2 \times 10^4 = 6.8 \times 10^5 \text{ C/m}^2$$

$$15.5 \text{ ml} = 3.7 \times 10^6 \text{ C}$$

Xenofused on 5/15/93
No transduced stem cells

| | | | |
|------|-----|------|----|
| G418 | 7ab | 1000 | 20 |
| - | 8ab | 2000 | 40 |

adsorb
from
fraction

Start:

 $5 \times 10^8 \text{ c}$ PRE
 0.71% Post ab
 0.22% *ul/ml media*34+ = $3.6 \times 10^6 \text{ c}$ = $1.1 \times 10^6 \text{ c}$ adsorbed
Fraction #1: $2 \times 10^6 \text{ c}$ FL1 FL2 gate
 31.94% FL1 FL2 gate
 20.81% *34+ = $0.64 \times 10^6 \text{ c}$ = $0.42 \times 10^6 \text{ c}$ adsorbed
Fraction #2: $0.8 \times 10^6 \text{ c}$ 2.46% 5.80% ± 34+c = $0.02 \times 10^6 \text{ c}$ $0.05 \times 10^6 \text{ c}$

(yields)

adsorbed #1:

$$\frac{\text{PRE} \& \text{FL1/FL2 gate}}{0.64 \times 10^6 \text{ c}} = \boxed{17.8\%}$$

$$\frac{\text{PRE} \& \text{FL1/FSC gate}}{0.42 \times 10^6 \text{ c}} = \boxed{11.7\%}$$

$$\frac{\text{post ab} \& \text{FL1/FL2 gate}}{0.64 \times 10^6 \text{ c}} = \boxed{58.2\%}$$

$$\frac{\text{Post ab} \& \text{FL1/FSC gate}}{0.42 \times 10^6 \text{ c}} = \boxed{38.2\%}$$

adsorbed #2:

PRE & FL1/FL2

PRE & FL1/FSC

removed from
medium

REMOVED

ZACHARY RIGGINS

5/14/93

REC'D 200 cc COLD BLOOD

PRE: $\frac{MORO}{109}$ \checkmark $\frac{POLY}{109}$

$$218 \times 50 = 10.9 \times 10^6 \text{ c/ml} \\ \times 200 \text{ ml} = 2.2 \times 10^9 \text{ c} \quad \text{start}$$

Added 3 vials (4.5 ml) 12.8 ab.
inc. 25 min.

Spun down. Rsp'd. in 1x PBS to 300 ml
in bag.

Ran through column.

Spun down unadsorbed fraction for 2nd ab
incubation.

Spun stem cell fraction to Rsp'd. in
smaller volume for count.

COUNTS:

unadsorbed

$\frac{MORO}{67}$ \checkmark $\frac{POLY}{102}$

$$10.9 \times 50 \times 10^6 \\ = 8.5 \times 10^6 \text{ c/ml} \times 225 \text{ ml} \\ = 1.9 \times 10^9 \text{ c}$$

stem

$\frac{MORO}{172}$ \checkmark $\frac{POLY}{16}$

$$188 \times 2 \times 10^4 \\ = 3.8 \times 10^6 \text{ c/ml} \times 55 \text{ ml} \\ = 20.7 \times 10^6 \text{ c}$$

inc
12.8
SPL
RST
Ran

COU

1

0

3

3.

=

Per
froz

com

26x1
won

= 2 =

13.51

LAON

incubated unadsorbed fraction w/ 4.5 ml
 12.8 ab. for 25 min.
 spun down.

Put in 300ml in bag (w/ 1x PBS)
 Ran through 2nd column.

counts:

unadsorbed
 monos polys
 30 33

$63 \times 53 \times 10^3$

$3.15 \times 10^4 \text{ cpm} \times 60 \text{ ml}$
 $= 1.9 \times 10^9 \text{ c}$

↓

stem
 monos polys
 58 4

$62 \times 2 \times 10^4$

$= 1.2 \times 10^6 \text{ cpm}$
 $\times 5 \text{ ml} = 6 \times 10^6 \text{ c}$

percolled/ficoll'd
 freeze \Rightarrow LOT (2)

combined stem cell fractions

$26 \times 10^6 \text{ c}$ for transduction

want final $[T] = 5 \times 10^4 \text{ cpm}$ \therefore

$= 2 = 200 \text{ ml supe}$

200 ml media

530 ml total

13 flasks 40 ml / flask

20 ml supe

20 ml media (B365)

+ 300 ml protamine sulfate

LAISON supe 539 (bottles 18/19)

$\times 10^4$
 ml $\times 55 \text{ ml}$

CFUs:

Set 745

PRE

| Plate # | Sample | # Cells | # ml |
|---------|-----------------------|-------------------|------|
| lab | (-G418) PRE + CFUs | 5.5×10^4 | 5 |
| 2ab | ↓ (+G418) | ↓ | 5 |

BBMM + 31615CF:

1L3 Sander # y0230292

1L6 Sander # y0450392

SCF AMGW # 150952

Took sample to micro for sterility ✓
 each day of transduction pt.
 Stat Gram stain done (negative)
 before cells were given to baby.

5/15/93 4pm 2nd transduction:

Spun cells down from each flask
 Respd in fresh media & L-ASN supe
 added Protamine sulfate

5/16/93 3rd transduction 330pm.
 Repeated above.

5/15

cell

Pre

Repl

Post

SG

+G4

SG

+G4

5117193 cells washed 4x
 3x in 1x PBS + Pls
 last wash in RPMI (no pls)

count: 60×10^6 c

$$\bar{x} = 15 \times 10 \times 10^4 = 1.5 \times 10^6$$

$$\times 40 \text{ ml} = 60 \times 10^6 \text{ c}$$

Put in 5cc into 10cc syringe

Reinforced on 5117193 (W5F)

Post-trans. CPUs: 59146

| sample plate # | | # cells | # ul |
|----------------|-----|---------|------|
| OG418 | 1ab | 500 | 4 |
| ↓ | 2ab | 1000 | 8 |
| ↓ | 3ab | 2000 | 16 |
| +G418 | 4ab | 500 | 4 |
| ↓ | 5ab | 1000 | 8 |
| ↓ | 6ab | 2000 | 16 |
| OG418 | 7ab | 1000 | 24 |
| +G418 | 8ab | 1000 | 24 |

ry ✓
 (412)
 > baby
 lion
 in flock
 SN supe

30pm.